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Potential of the Sebia Capillarys® neonat fast automated system for neonatal screening of sickle cell disease

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Abstract

Background: Most screening programs for sickle cell disease (SCD) utilize isoelectric focusing (IEF) or high performance liquid chromatography (HPLC) to detect haemoglobin (Hb) variants. The first method is not automated and becomes too tedious when many samples have to be investigated. The aim of this work is to explore the capacity of an automated capillary electrophoresis (CE) system, with full traceability, as a tool for newborn screening of SCD.
Methods: The Capillarys® neonat fast automated system has been developed by Sebia for newborn screening. We performed separate studies using different types of samples to evaluate the utility of the Capillarys® for (i) separating Hb S and other variants, and (ii) for performing the routine activity of our laboratory for 20 working days.
Results: A throughput of 48 samples per hour with a loading capacity of 192 samples was achieved. Migration times of the major Hb variants were distinct. There were few variants showing similar migration times to Hb S and Hb C and thalassaemia could be detected. In addition, late screening, screening of premature or transfused babies and screening performed using poor quality Guthrie's cards did not interfere with reporting of accurate phenotypes.
Conclusions: Sebia Capillarys® neonat fast automated system is a reliable tool for haemoglobinopathy neonatal screening.
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Keywords: capillary electrophoresis; newborn screening; sickle cell disease; thalassaemia.

Introduction

The sickling of red cells may result from inheritance of the gene for sickle haemoglobin (HbS) associated to another inherited gene for either HbS, or an interaction with another haemoglobin (Hb) such as HbC, HbD-Punjab, HbE, HbO-Arab, HbLepore, HbG-Philadelphia or with β-thalassaemia (1). The strategy developed in France for newborn screening for sickle cell disease (SCD) is a combination of two methods (2): first, isoelectric focusing (IEF) used for primary screening is rapid but not-automated; second, a second tier test incorporates high performance liquid chromatography (HPLC) with a cation exchange column. The latter is automated, fast and quantitative. The first step can become very tedious and time-consuming when many samples need to be evaluated (100,000 newborns are tested each year in our screening laboratory). A technical improvement is necessary for screening centres with high testing volumes. The aim of this work is to explore the utility of capillary electrophoresis (CE) as a powerful tool for SCD newborn screening. The analytical challenge of this work is the ability to detect all the haemoglobinopathies able to induce a sickling of red cells, as well as other variants of interest such as HbBart's (α-thalassaemia marker), whatever the condition of the blood sample. Two previous studies published by Gulbis et al. (3) and by Louahabi et al. (4) reported that ''capillary electrophoresis, due to its high analytical efficiency, can replace conventional techniques'' for detecting haemoglobinopathies. These studies used dried blood samples from Guthrie cards with normal and abnormal Hbs that were tested using the new Capillarys® neonat fast technology. They offer the first prospective evaluation of the Sebia Capillarys® neonat fast automated electrophoresis system applied to SCD newborn screening. Its performance was compared to well established methods (IEF/HPLC).

Our laboratory, located at the University Hospital of Lille (France), is one of four accredited French Centres for SCD neonatal screening. Two-thirds of newborns in France (Paris region and Southern area excluded) are analysed by our centre. In addition, SCD screening for French Guiana, Reunion Island and Mayetta Island newborns is also performed by our laboratory. Consequently, in our study not all dried blood samples were of high quality. Guthrie cards for routine screening activity of our laboratory were investigated. Some came from remote regions such as French Guiana. These samples might be several weeks or months old and kept for 2–3 weeks in a tropical atmosphere before they were sent to our laboratory. Thus, the Capillarys® neonat fast automated system was tested under extremely difficult conditions.

Materials and methods

Materials

The automated electrophoresis system Capillarys® 2 (Sebia, Evry, France) was modified in order to improve its loading

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capacity and analysis throughput. Specific accessories are described in Figure 1.

Capillary electrophoresis

The pre-analytical step Demineralised water was added with a multipipette into the wells of the Capillarys® segment for pre-elution of Hb; 3.8 mm spots from the Guthrie cards were obtained using a BSD Duet® puncher (BSD Robotics, Queensland, Australia). They fell directly into the wells of the Capillarys® segment. The holder moves on an x and y axis according to the thread between the wells from a segment and the thread between the segments present on the holder. The barcode for each sample and the Guthrie card barcode for each dilution segment are scanned using two specific barcode readers. A pre-elution time from 2 h up to 3 days at 2–8°C in a humidity chamber, without shaking, was applied. To prepare the racks, a segment is set on a rack, and a tube containing haemolysing solution is inserted. Racks can be introduced successively and continuously.

The analytical step (inside Capillarys® neonat fast automated system)

- Automated reading of segment and rack barcodes
- Automated addition of haemolysing solution to the pre-eluted blood spot samples
- Automated mixing in the wells
- Automated injection without any manual transfer of the haemolysates into the capillaries
- Eight parallel capillaries are used to analyse the samples at 10,000 V for 7 min at a temperature of 34°C with an alkaline buffer (pH 9.4)
- Direct measurement of Hb at 415 nm
- Curves are automatically displayed on the screen and identified by the sample barcode

A throughput of 48 samples per hour was achieved. Up to 24 sample racks (192 samples) can be introduced at one time.

The post-analytical step The different migration zones of Hb variants (termed N1–N13) were indicated on the electrophoreticgrams. Passing the cursor over a particular zone displays icon information containing the possible Hb variants that might be seen in this zone. Abnormal curves (in red) are automatically discriminated from normal curves (in blue).

Sample collections

Five hundred and sixty-four Guthrie cards with residual dried blood spots were sequentially selected by our laboratory. The corresponding heel prick samples were collected between 3 and 5 days after birth onto Waters grade 903 filter paper. Birth weight and gestational age were recorded for each newborn. Four hundred and eighty-five of these newborns had a relatively common abnormal Hb. The other samples were considered to be normal. One hundred and six premature newborns with normal or abnormal Hb were included in this cohort of 564 samples. This first sample collection was investigated retrospectively in Sebia's laboratories which performed a repeat analysis after the screening laboratory in Lille (France) had performed its analysis. The delay between the analysis by IEF/HPLC and the analysis by the Capillarys® neonat fast automated system was three weeks in the majority of cases, but never exceeded three months. During this time, the samples were kept at +4°C. Samples from newborns from French Guiana, Reunion

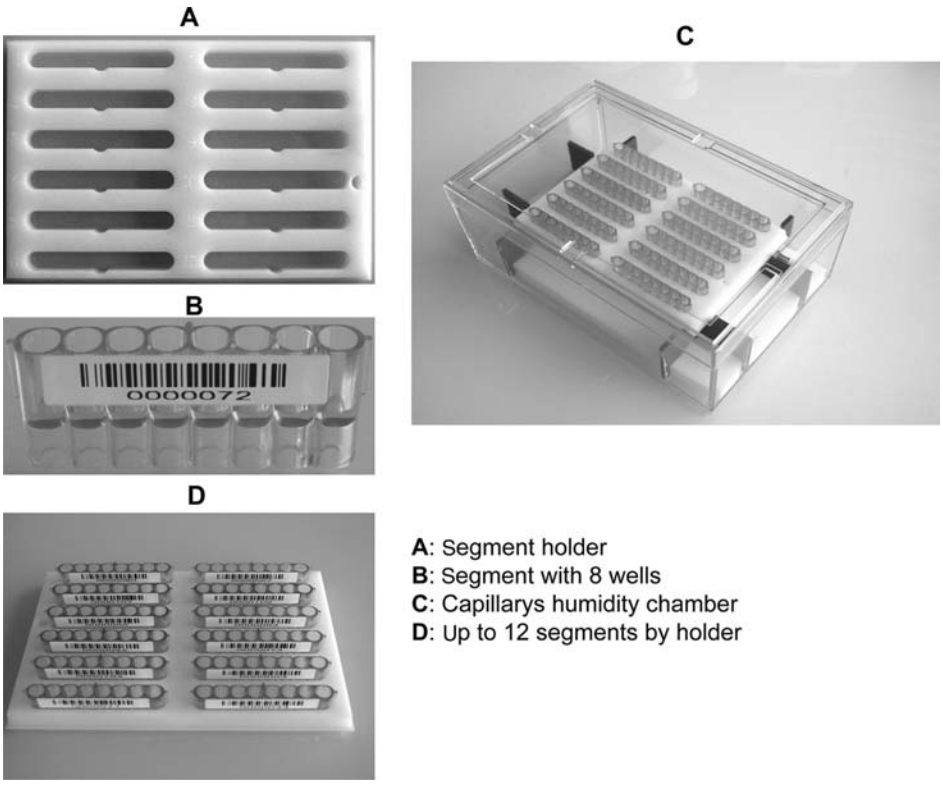

Figure 1 Materials supplied by Sebia (Evry, France) with the Capillarys® neonatal fast electrophoresis apparatus. Segment holders (A); segments identified with barcode (B); humidity chamber (C). Segments and segment holder assembly can be viewed in (D). Guides located on each side of the segments are useful for maintaining them correctly on the holder.

Island and Mayetta Island were excluded from this part of the study. Comparison between the two screening strategies was performed in blinded fashion.

In the second part of this study, we compared the Capillarys® neonat fast automated system to the established technique of IEF. All cards received each working day during 4 weeks were tested in parallel with both methods in our screening laboratory, and using the same staff. IEF was performed first followed by CE the next day. This second sample collection contained more normal Guthrie cards compared with cards obtained with the first collection. This was used to evaluate the possible integration of the Capillarys® system into routine newborn screening for SCD. These samples originated either from metropolitan France (4307), Reunion Island (957), French Guiana (291) or Mayetta Island (647).

All samples were collected with consent from the parents.

Method reliability

Different neonatal samples were chosen: samples A and B = normal samples; samples C and D = pathologic samples (with HbA and HbS); sample E = a series of eight samples (4 normal, 3 HbS heterozygotes, 1 HbBart's).

Within-run reproducibility Each sample from A to D was analysed eight times the same day and by the same technician.

Between-run and between-lot reproducibility Three runs with the series of sample E were performed with one lot of buffer and haemolysing solution. This study was completed using 2×3 runs with two additional lots of buffer and haemolysing solution.

No ethical approval was required since the Capillarys® has been evaluated using residual dried blood and this automated system has been used exclusively to perform screening for SCD. In France, all samples for newborn screening are collected with consent of the parents.

Results

The two tier procedure (IEF/HPLC) used as the reference method for screening of SCD in our study provides putative identification of clinically significant haemoglobinopathies and the risk of errors is reduced (2). Genotyping should be performed following the first consultation with a paediatrician in order to establish a definitive diagnosis.

Retrospective analysis of the first cohort

Normal and abnormal patterns The newborn shown in Figure 2A is normal, while the newborns shown in Figure 2B and C show patterns frequently observed in newborn screening: Figure 2B shows the profile of a newborn that received a blood transfusion and Figure 2C shows the profile of a very low birth weight preterm infant. Normal Hbs HbA and HbF (zones N10 and N7, respectively) were clearly identified in normal newborns (Figure 2A). HbA2 (zone N2) was clearly distinguishable in the profile from the baby that had been transfused (Figure 2B). Only in extremely preterm infants was the amount of HbA too low to form a sharp peak (Figure 2C). A premature baby (Figure 2C) can nevertheless be differentiated from a newborn presenting with β°-thalassaemia: in the absence of HbA (Figure 2D), a small round peak corresponding to acetylated HbF was located exclusively in zone N9, and no HbA peak can be seen in N10; when a small amount of HbA was mixed with acetylated HbF (similar to premature newborn in Figure 2C), the resulting large round peak was present and borderline between zones N9 and N10 (i.e., detection of peaks in N9 and N10).

This cohort included 28 newborns of normal weight (>2500 g) and a term >37 weeks. Some profiles from normal newborns deviated from the expected normal pattern. A large peak in N10 (HbA) was observed in two cases where preterm infants had been transfused. A minor HbA peak in N10 or a large round peak located between N9 and N10 was seen in 52 cases from non-transfused preterm babies.

Newborns with HbS variant In Figure 3A, a HbS peak observed in N4 was associated with a small round peak in N9 and no peak in N10. This indicated a F/S homozygous profile. The Capillarys® system was sensitive enough to detect SCD in a 34-week preterm newborn (Figure 3B). In Figure 3C, there is no HbA in N10, a peak of acetylated HbF in N9, and the presence of HbS and HbC (respectively in N4 and N1). This indicated a F/SC compound heterozygous profile. These three newborns with abnormal profile were identified easily and not confused with a HbS carrier (Figure 3D).

Among samples obtained in the first collection, there were 47 newborns with SCD. Forty were FS homozygotes and seven were F/SC compound heterozygotes, two of which were preterm infants. There were 307 term newborns and 37 preterm infants showing a F/AS heterozygous profile. The concentration of HbS measured by HPLC in premature newborns who were homozygous or heterozygous varied from 0.8% to 4.2%. All were interpreted correctly according to Capillarys® profiles.

Newborns with HbC, D-Punjab or E variants Figures 4 and 5 were interpreted according to the same scheme: (i) a homozygous profile showing the characteristic variant peak (HbC in N1, Figure 4A; HbD-Punjab in N5, Figure 5A; HbE in N3, Figure 5C), and no peak in N10 in the absence of HbA. (ii) A heterozygous profile (Figures 4B, 5B and D) with a characteristic variant peak and a sharp peak in N10 indicating the presence of HbA. (iii) A profile from a preterm newborn (Figures 4C and 5E) with a small variant peak and a round peak that borders N9 and N10, indicating the presence of HbA in small amounts. No F/AD premature newborns were found in this cohort.

Among the 564 selected samples, the two newborns showing a F/C homozygous profile were identified correctly. The numbers of HbC, HbD-Punjab or HbE carriers were 53 (including 8 preterm infants), 12 and 19 (including one premature baby), respectively.

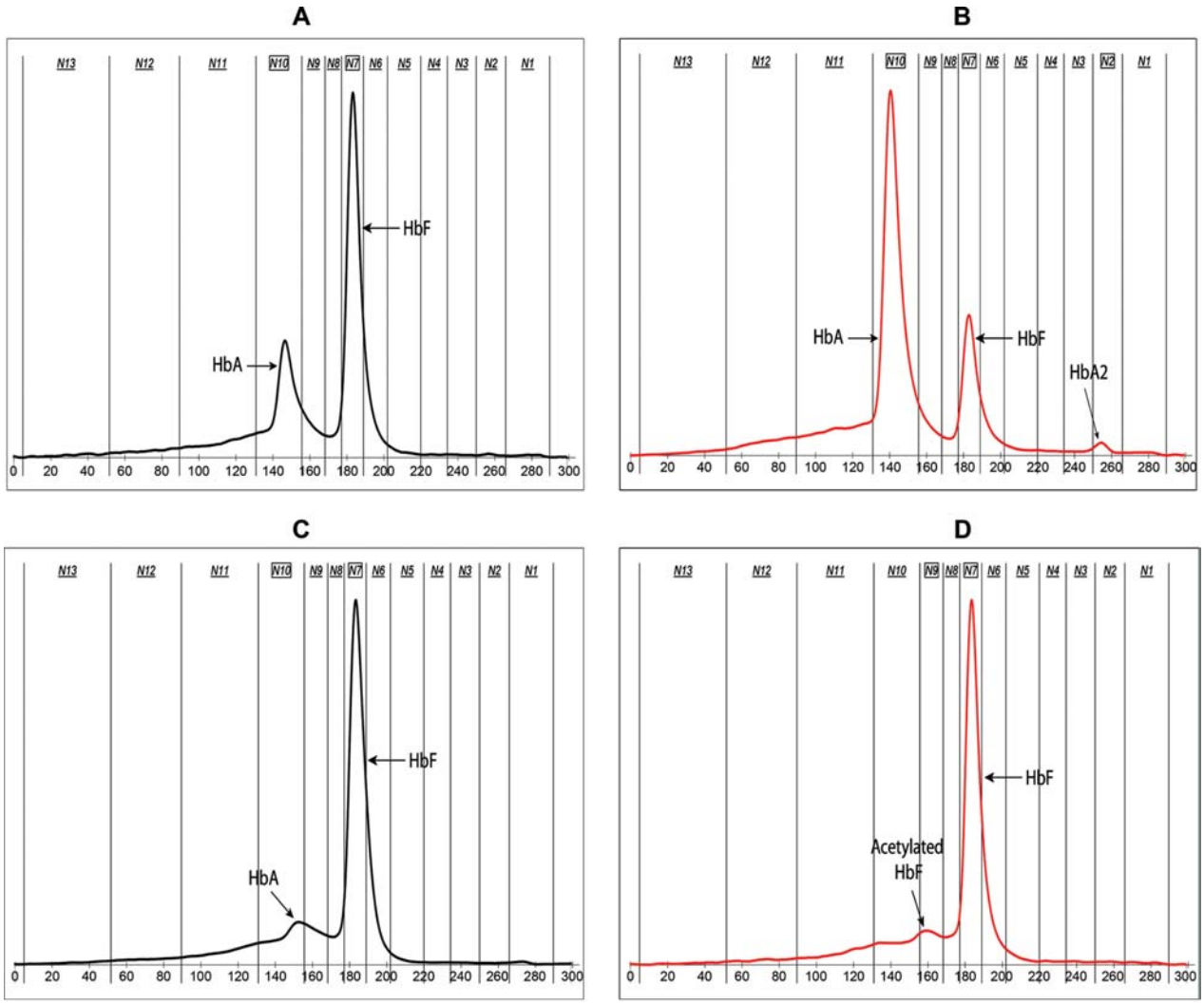

Figure 2 Newborns without haemoglobin variants.
(A) Normal newborn: 39 w. term; 3445 g. (B) Transfused newborn: 38 w. term; 3100 g. (C) Premature newborn: 30 w. term; 1060 g. (D) Term newborn with β°-thalassaemia.

Newborns with less common β-chain variants HbKorle-Bu and HbO-Arab variants were selected because they may have an impact on the specificity of SCD screening. These two variants migrated in N5 and N2, respectively (Figure 6A and B).

Two newborns with F/AKorle-Bu, 5 with F/AO-Arab and 1 F/O-Arab homozygote were also included in this cohort. Electrophoreticgrams showed good agreement with IEF/HPLC.

Newborns with γ- and α-chains variants Three profiles were observed most frequently: (i) a single peak detected in N3, higher than HbA (Figure 6C). On the corresponding HPLC profile, one major peak migrated between HbF and HbA. This fraction usually corresponded to HbF-Ouled Rabah; (ii) two abnormal peaks were detected. A major peak and a minor peak located in N3 and N5, respectively. The intensity of the first one was higher than HbA (Figure 6D). Using HPLC, a minor peak with the same retention time (RT) as HbD-Punjab was observed, in addition to a major peak between HbF and HbA. This profile suggests the presence of HbG-Philadelphia and is in agreement with IEF; (iii) similar to the second pattern but with peaks in N11 and N12 (Figure 6E). These eluted using HPLC as two peaks, weakly retained by the column, and were considered to be ''fast-moving'' foetal Hb variants.

All 23 putative variants of γ-/α-chains present in our cohort were detected by the Capillarys® neonat fast automated system.

Newborns with α-thalassaemia Newborn screening for SCD allows the opportunity to detect Hb H disease. Twenty-one newborns, including six preterm infants, presented with a minor HbBart's peak migrating in N13 (Figure 7A), corresponding to single or double α-chain deletion. Two cases of HbH disease (triple deletion of α-chain) presented with a marked peak in N13 (Figure 7B).

Newborns with β-thalassaemia The Capillarys® profiles from four babies showed only a small round peak in N9, indicating the absence of HbA and a risk of β°-thalassaemia. These results were in agreement with IEF/HPLC. In two other cases, a large round peak

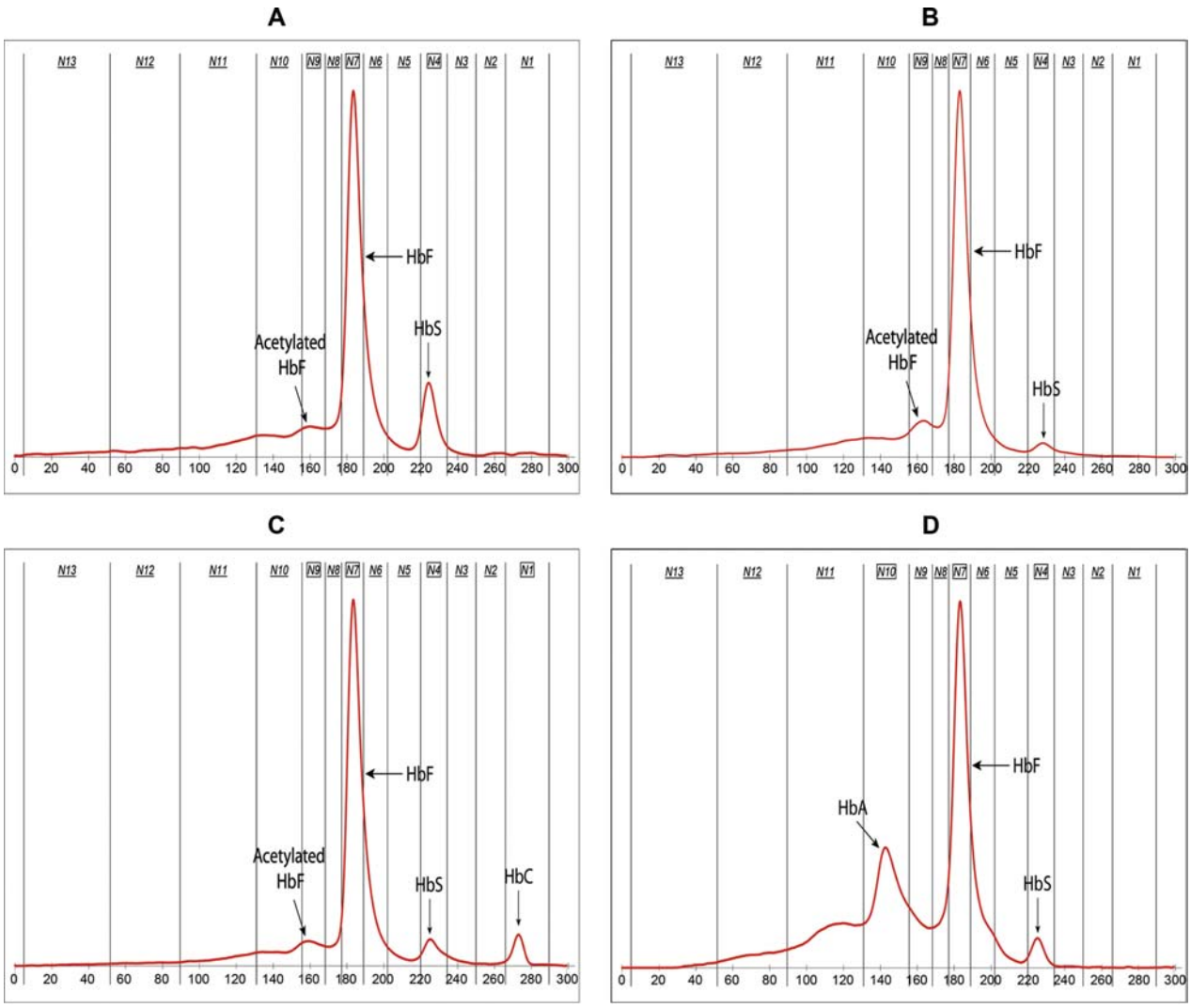

Figure 3 Newborns with haemoglobin S.
(A) F/S homozygote: 37 w. term; 3490 g. (B) Premature F/S homozygote: 34 w. term; 1300 g; quantification of HbS by Variant I from Bio-Rad (Bio-Rad Laboratories, Hercules, CA, USA) has given a relative concentration of 1.2%. (C) F/SC compound heterozygote: 40 w. term; 3900 g. (D) F/AS single heterozygote: 39 w. term; 3180 g.

was present that was borderline between N9 and N10, and was associated with an HbS variant in N4. This type of profile was consistent with HbS/β^+-thalassaemia (Figure 7C).

Prospective analysis of the second cohort

SCD screening with the Capillarys® neonate fast automated system and the BSD Duet® Puncher One Capillarys® neonat fast automated system and one BSD Duet® Puncher were provided by Sebia for 20 working days. We analysed 6202 Guthrie cards. To achieve the throughput of 350 samples per day, the less fresh Guthrie cards were punched and pre-eluted overnight. The next morning, 24 racks with the pre-eluted samples were loaded into the CE apparatus. Analyses were completed 4 h later. Three hours prior to the second loading, and in order to let enough time for punching and pre-elution, 24 new segments were prepared.

To utilize the Capillarys® neonat fast automated system at full capacity of 576 samples per day, a supplementary cycle of punching and pre-elution of blood spots (24 segments) needed to be planned during the analysis time of the second series for overnight analyses.

Efficiency of automatic discrimination of normal and abnormal profiles A profile was considered normal when HbF and HbA peaks were present. A profile was considered abnormal and displayed as a red curve when the following criteria were met: (i) the absorbance of HbF peak was very low [optical density (OD) <0.0018 in N7]. In these cases, the migration zones are not indicated on the profiles; (ii) no peak was distinguished in N10, which implied no HbA; (iii) additional peaks were present in other zones. Their detection was due to an OD higher than a threshold defined for each zone when compared to the background.

Among the 6202 samples tested in the second cohort, 549 profiles (9%) were automatically classified by the Capillarys® using a red colour. In practice, this

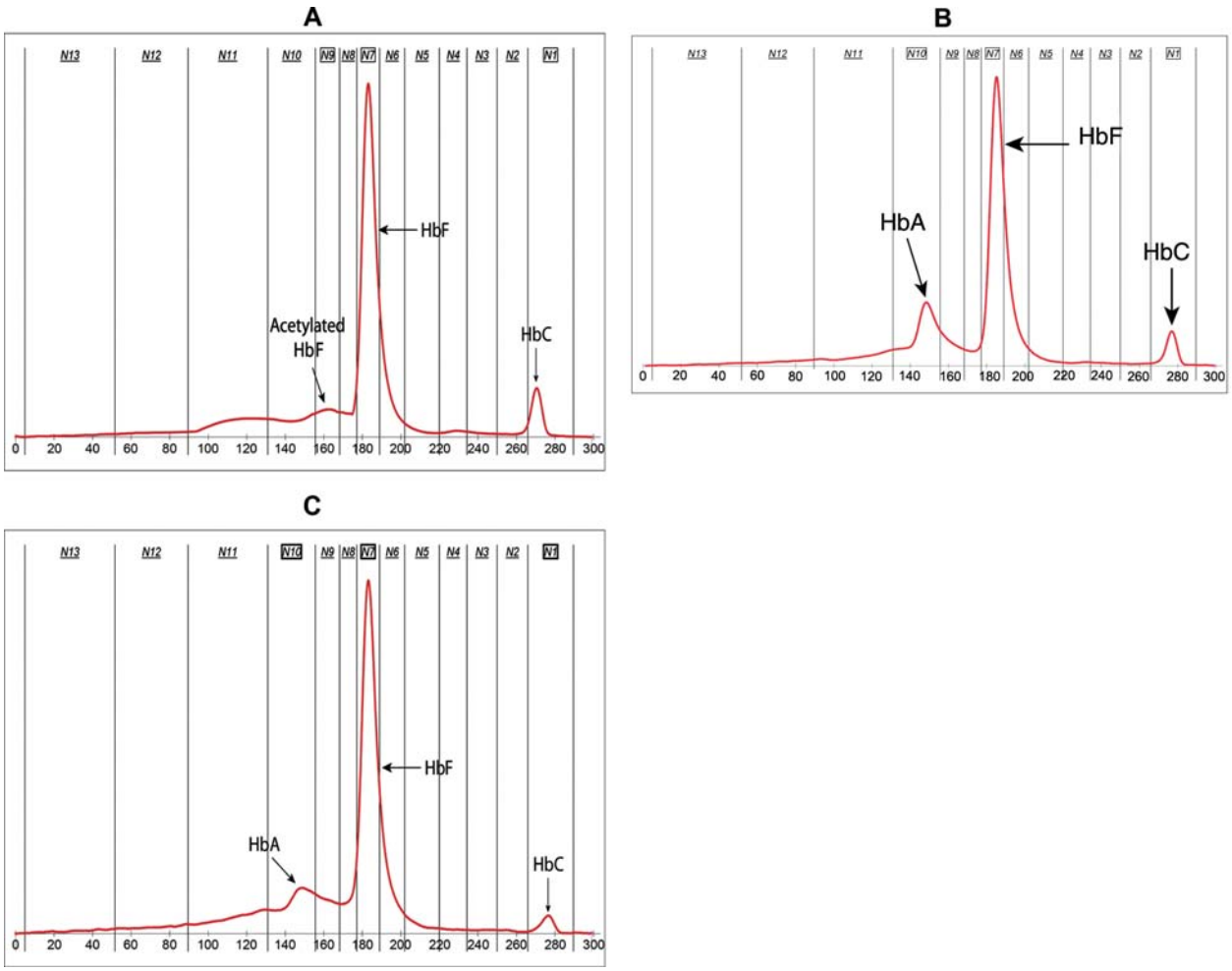

Figure 4 Newborns with haemoglobin C.
(A) F/C homozygote: 39 w. term; 3490 g. (B) F/AC heterozygote: 40 w. term; 3035 g. (C) Premature F/AC heterozygote: 30 w. term; 2330 g; HbA (HPLC) = 1.5%; HbC (HPLC) = 1.2%.

labelling of abnormal profiles was activated in four scenarios:

i. The presence of a distinct HbA2 peak in N2 associated with an increase in the amount of HbA. We found 118 samples with this profile in this cohort. Twenty were found in newborns screened outside the usual prescribed time. The intensity of the HbA peak was equal to HbF (4 cases) or higher (2 cases). It is interesting to note that some pitfalls can occur when screening is delayed (2–3 months after birth). The migration zones were not indicated, despite migration of HbA and HbF at appropriate time (10 cases), or HbA/HbF were incorrectly identified as HbF/HbS (4 cases). In one extreme situation where the infant was tested when nine months of age, a major peak observed in N7 was confused with HbF.

   For 93 full-term newborns, the HbA peak was as large as the HbF peak, or even greater, although these newborns were tested 3–5 days after birth. This type of profile could be the consequence of early switching from HbF to HbA (5). A low level of HbF at birth, as observed in γ-thalassaemia syndromes (6), could also explain this type of profile. Since full-scale display was always applied to HbF, the size of the HbA and HbA2 peaks consequently appeared increased.

   The last five cases corresponded to newborns that had been transfused as documented in the medical record. These newborns showed high amounts of HbA and very low amounts of HbF.

ii. The absence of HbA. In some cases, HbA could not be detected by the Capillarys®. The best illustration of this problem is illustrated by comparing Figure 2C and D; the former is in black and the latter in red. No cases of β°-thalassaemia were present in the second cohort.

iii. The detection of Hb variants. The areas of interest were zones N1–N6 and N11, N12, N13. The N1 zone was the migration area for HbC. Thirty-three newborns, including three premature babies, were correctly identified as heterozygotes FAC. The N2 zone was shared by HbA2 and HbO-Arab. A carrier of this β-globin variant was found in a term newborn. In the N3 zone several variants were confirmed by IEF/HPLC: HbE in seven cases, HbF-Ouled Rabah in one case, HbG-Philadelphia heterozygotes in three cases. Two other samples were assumed to contain unidentified variants. HbF-Ouled Rabah can be differentiated from HbE

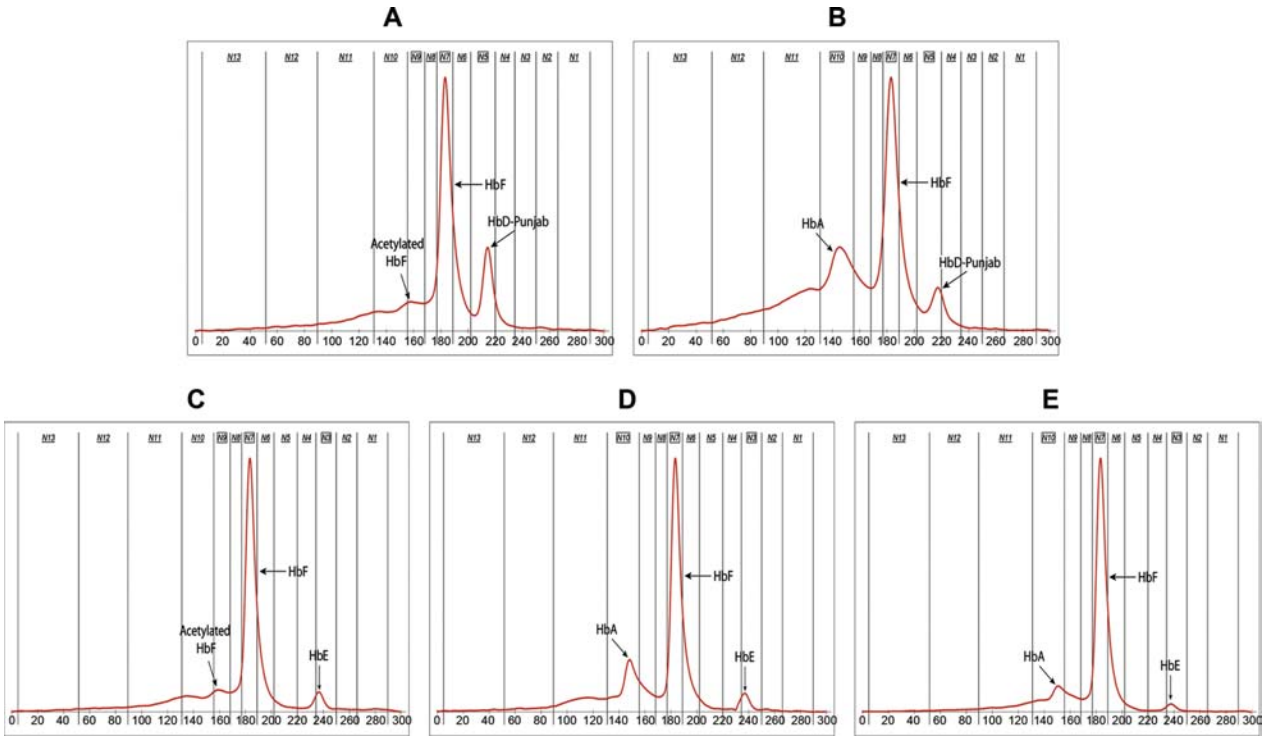

Figure 5 Newborns with haemoglobins D or E.
(A) F/D homozygote. (B) F/AD heterozygote: 39 w. term; 3270 g. (C) F/E homozygote: 40 w. term. (D) F/AE heterozygote: 39 w. term; 3420 g. (E) Premature F/AE heterozygote: 35 w. term; 1840 g.

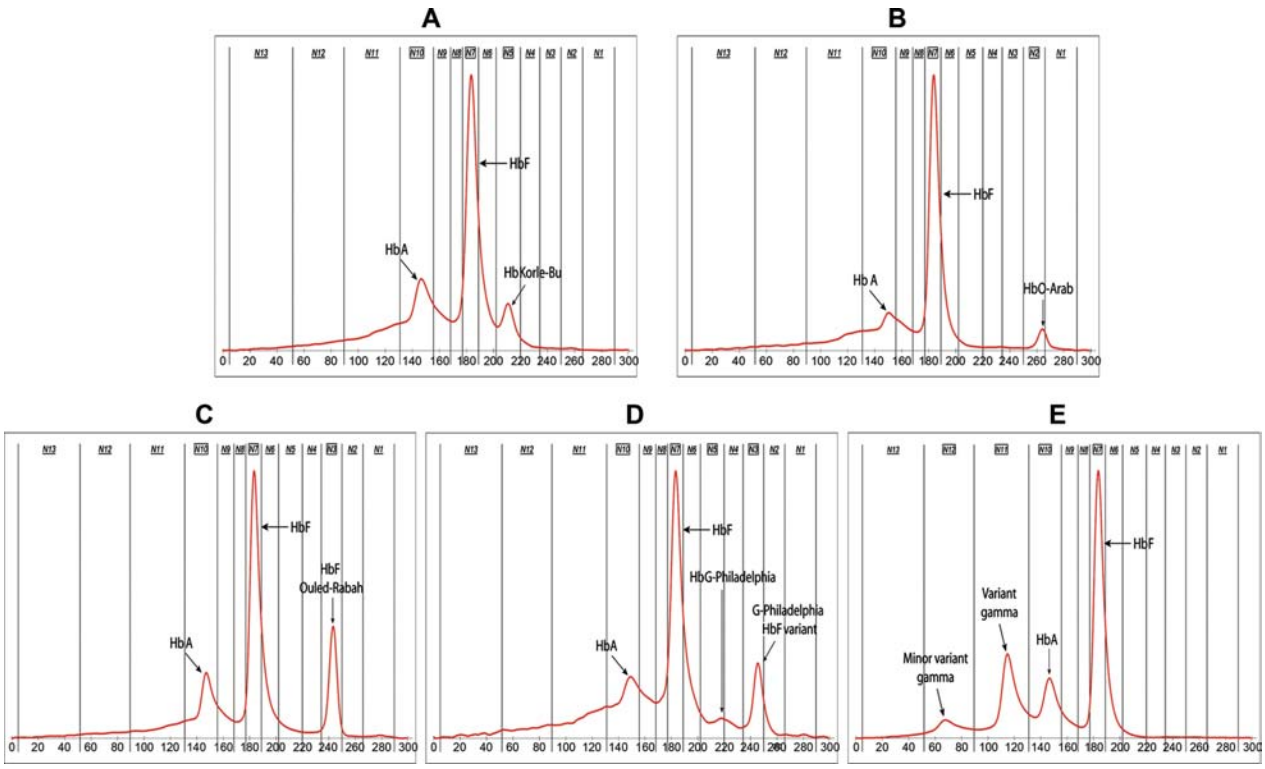

Figure 6 Newborns with rare haemoglobin variants.
(A) F/AKorle-Bu heterozygote: 40 w. term; 3790 g. (B) F/AO-Arab heterozygote: 40 w. term; 3120 g. (C) FA/F-Ouled Rabah heterozygote: 37 w. term. (D) F/AG-Philadelphia heterozygote: 39 w. term; 3490 g. (E) Fast moving foetal haemoglobin variants. Premature baby.

on the basis of its thinner shape and intensity, which is higher than that of HbA. Likewise, HbG-Philadelphia can be directly identified because the major peak in N3 was always associated with a minor peak in N5. In the N4 zone, the main variant was HbS: one FS homozygote and 155 FAS het-

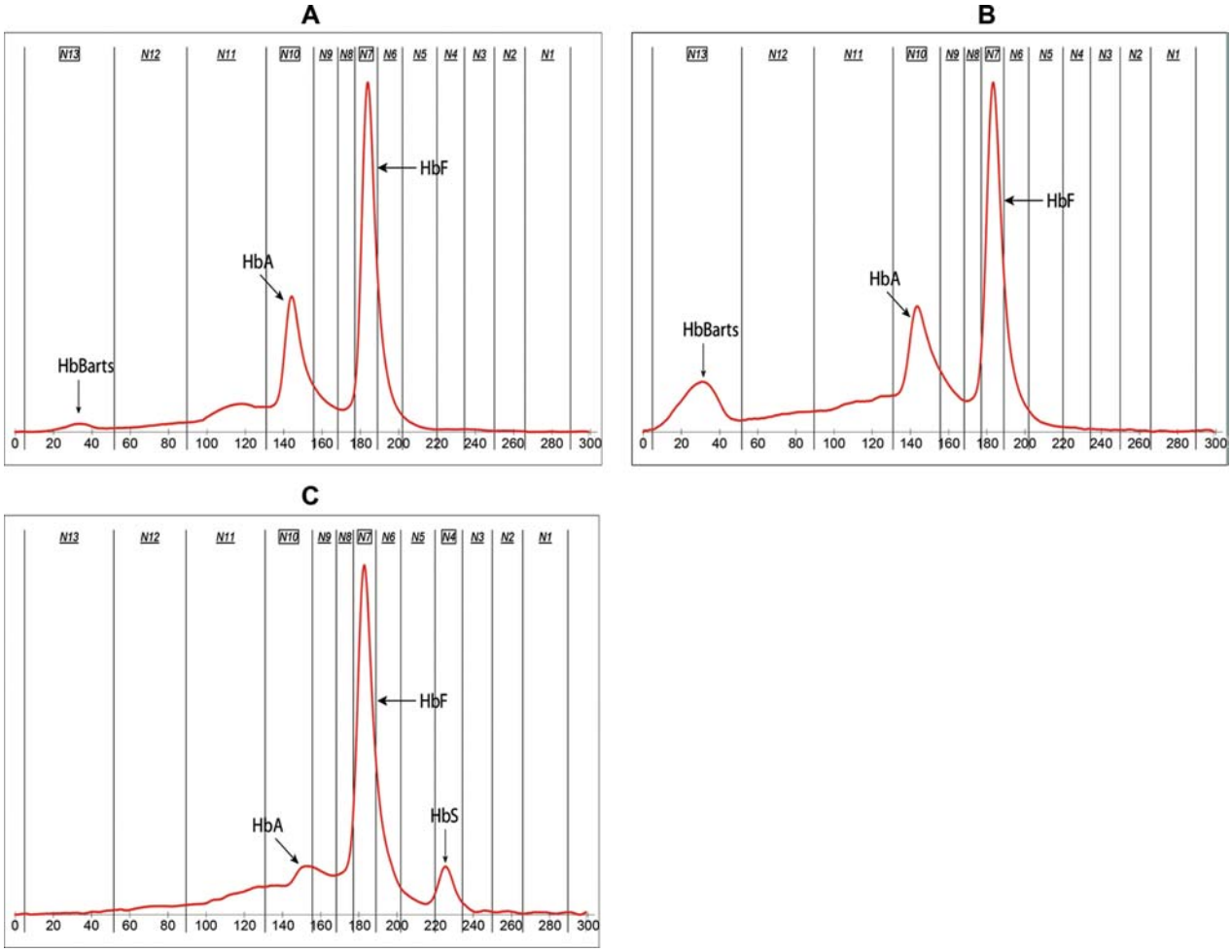

Figure 7 Newborns with α-thalassaemia and β-thalassaemia.
(A) Minor HbBart's: 38 w. term; 2790 g. (B) Major HbBart's: 39 w. term; 2400 g. (C) F/AS heterozygote with β^+-thalassaemia (S/β^+-thal compound heterozygote).

erozygotes were detected and confirmed with HPLC. Four discrepancies were observed: IEF/HPLC results were suggestive of an α-/γ-variant, or the variant detected by IEF and Capillarys® was not revealed using HPLC. From 155 FAS profiles, 28 newborns (including 10 premature) were screened outside the prescribed time. In the N5 zone, HbD-Punjab and HbKorle-Bu were determined and only one heterozygote of each was found. In the N6 zone, an unidentified variant was discovered. Its HPLC profile revealed a faint peak in proximity to HbA. In the N11/N12 zones, we found three fast-moving foetal Hb variants. A minor and a major peak were found, respectively, in N12 and N11 in two newborns. Their HPLC profiles showed two peaks with RTs = 0.22 and 0.50 min, respectively. A third variant migrated alone in N12 (RT = 0.82 min). In the N13 zone, low amounts of Bart's Hb were detected in 88 cases, including four associated with HbS. A very fast-moving variant was detected in N13 which corresponded to two peaks by HPLC (RTs = 0.17 and 0.25 min).

iv. The detection of altered profiles. The poor quality of the corresponding samples did not cause false negatives: five FAS, one FAC heterozygote and 36 with increased concentrations of HbA were correctly identified.

Fourteen profiles had a very noisy background from N13 to N1. The common feature shared by these samples was a period of 10–45 days between sample collection and analysis.

Most of the profiles presenting with a noisy background were restricted to one or several migration zones. False positives occurred most frequently in N11 (41 cases), N13 (27 cases), N1 (15 cases), N11 + N13 (3 cases), N12 + N13 (4 cases), and N1 + N11 + N13 (24 cases). Among these 114 altered profiles, 78 were performed late, between 8 and 25 days following blood collection, and 72 came from tropical areas as Mayetta Island/Reunion Island/French Guiana and might have been subject to rapid ageing due to storage and travel conditions.

Reliability

The detection of the Hb variants and the automatic labelling to sort abnormal profiles were not modified from one run to another. The expected peaks were

observed in the correct zones without contamination of the other zones by unidentified material.

Discussion

The Hb genotypes from the dried blood cards used to evaluate the Capillarys® neonat fast automated system have been determined by integrating data from IEF and HPLC, without β-globin gene sequencing. This means that no ''confirmation'' is provided by the combination of these methods. Thus, HbS variants are putatively identified until family studies have confirmed this common variant by a sickling test or molecular analysis. In addition, patterns such as S/S, C/C, E/E, D/D cannot be distinguished from hemihomozygous syndromes with β°-thalassaemia, which is quite important for HbE/β-thal and C/β-thal. Homozygous HbE may not have clinical significance, but the interaction of HbE with thalassaemia produces variable phenotypes ranging from mild to severe that simulate homozygous β-thalassaemia (7). Homozygous HbC results in a compensated haemolysis (8). However, HbC/β°-thalassaemia is more severe and can mimic β-thalassaemia intermedia (9). These drawbacks of SCD screening using IEF and HPLC are not resolved with CE.

In the first cohort, characteristic profiles were obtained for preterm newborns and four cases of β°-thalassaemia were recognised along with two cases of β^+-thalassaemia associated with HbS. The possibility of detecting β^+-thalassaemia or very low weight premature infants by automatic labelling should be possible as soon as the quantification of Hb fractions is available. The method presented could lead to some rare initial misinterpretations with extreme preterm infants. The HbA peak is rather round and not as sharp as that obtained by HPLC. However, its differentiation from the β°-thalassaemia profile, characterised by the presence of acetylated HbF, was possible. Inconclusive samples that are verified by HPLC should be genotyped. Although rare compound heterozygous phenotypes such as HbD-Punjab, HbE, HbO-Arab or HbG-Philadelphia interacting with HbS were not seen in and of the newborns tested, the corresponding single heterozygote or homozygote conditions were correctly identified by the Capillarys® neonatal fast automated system. Minor and major HbBart's were also detected. This technique is also able to identify HbLepore (10). False positives found classically by IEF (2) are not seen with CE. Samples with HbC/HbKorle-Bu should not be misinterpreted as heterozygous HbS/HbC. Similarly, with CE it is easy to differentiate HbS/HbC from a HbS/HbE heterozygote or a HbS carrier from HbD-Punjab, or a HbC carrier from a HbE carrier. Also, HbC and HbO-Arab are clearly separated by the Capillarys®, whereas these two variants are not discriminated using IEF. In addition, rare α-globin or γ-globin variants give clear profiles that are easily identified.

With the second cohort, we tested 6766 specimens with the Capillarys® automated system. The first major point that we clarified is that three series of 192 samples can be analysed per day. The purchase of two Capillarys® neonat fast automated system was optimal for performing screening for SCD in 100 000 newborns per year. If we compare CE to IEF/HPLC, an efficient technician could analyse a maximum of 720 samples per day by IEF. However, the technician had no ''free time'' and was fatigued. The use of a Bio-Rad Variant Newborn Screening System with three HPLC modules had been recommended by this company for automation of our SCD screening program (personal communication).

The second major point was evaluation of the efficiency of automated discrimination of normal profiles from abnormal profiles. No marked discrepancy was seen between this system and IEF. All newborns presenting with SCD were identified. The percentage of automatically selected profiles is acceptable for the first step of SCD screening, and is no higher than the percentage of abnormal profiles shown by IEF. Interferences from Hb variants that migrate similar to HbS and HbC are relatively rare. Only very fast-moving foetal Hb variants may be confused with HbBart's. In addition, late screening of premature babies and screening performed using Guthrie cards of poor quality did not markedly interfere with reporting of the correct phenotype. However, some pitfalls can occur. No identification of migration zones was the most common issue. This occurs when analysis is performed very late using Guthrie cards that are eluted with more difficulty. However, this threshold is sometimes too high since HbF can occasionally be confused with HbA. In these cases, HbA/HbF may be misinterpreted as HbF/HbS. The possibility of such false positives needs to be known, and the time between birth and analysis by CE must be taken into consideration during validation of abnormal profiles.

This study explores a stepwise strategy for neonatal screening of SCD. It combines the Capillarys® neonat fast automated system with second tier-testing by HPLC. This strategy maintains the same specificity and sensitivity as IEF/HPLC, but offers high throughput and the advantages of more efficient automation of the first step, full traceability, and help with interpretation (automatic discrimination of abnormal samples, detection of HbBart's, an on board library of variants). In addition, quantitation will be soon available with ratio calculations between HbA and any abnormal fractions. This will help the biologist to make the diagnosis of β-thalassaemia.

Detailed description of the different haemoglobin variantes cited in the text

HbF, foetal haemoglobin composed of α- and γ-chains ($\alpha_2\gamma_2$); HbA, adult haemoglobin composed of α- and β-chains ($\alpha_2\beta_2$); HbA2: minor adult haemoglobin composed of α- and δ-chains ($\alpha_2\delta_2$); HbS, variant S of the β-chain ($\alpha_2\beta^S{}_2$); HbC, variant C of the β-chain ($\alpha_2\beta^C{}_2$); HbD-Punjab, variant D-Punjab of the β-chain ($\alpha_2\beta^{D\text{-}Punjab}{}_2$); HbE, variant E of the β-chain ($\alpha_2\beta^E{}_2$); HbO-Arab (not Hbo), variant O-Arab of the β-chain ($\alpha_2\beta^{O\text{-}Arab}{}_2$); HbKorle-Bu, variant Korle-Bu of the β-

chain ($\alpha_2\beta^{Korle\text{-}Bu}{}_2$); HbLepore, haemoglobin composed of 2 normal α-chains and 2 δ-β-fusion chain; HbG-Philadelphia, varient G-Philadelphia of the α-chain; HbF-Ouled Rabah, variant Ouled Rabah of the γ-chain; γ variants, variants of the γ-chain; α variants: variant of the α-chain; FS, newborn homozygote for the variant S of adult haemoglobin; FAS, newborn heterozygote for the variant S of the adult haemoglobin; FAC, newborn heterozygote for the variant C of the adult haemoglobin; FSC, newborn compound heterozygote for the variants S and C of the adult haemoglobin; α-thalassaemia, no synthesis of α-chain; Hb Bart's, Hb composed by four γ-chains (γ_4); β°-thalassaemia, no synthesis of β-chain; β^{+}-thalassaemia, decreased synthesis of β-chain.

Authors' disclosures of potential conflicts of interest

No authors declared any potential conflicts of interest. Sebia Company played no role in the design of our study, choice of enrolled patients, review and interpretation of comparative data. Moreover, the authors have not accepted any funding or support from an organization that may in any way gain or lose financially from the results or the conclusions of this article. No author was ever employed by an organisation that may in any way gain or lose financially from the results or the conclusions of this study.

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